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Gene expression of transforming growth factor-β receptors types I and II in rat endometrium during the estrous cycle and early pregnancy

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Abstract

Roles of transforming growth factor-β (TGF-β) receptor types I (TβRI) and II (TβRII) during the estrous cycle and implantation of rodents are currently unclear. In the present study, the spatial and temporal expressions of TβRI and TβRII in rat endometrium during the estrous cycle, pre-, and peri-implantation were examined using in situ hybridization and semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). With in situ hybridization, TβRI and TβRII were expressed at weak levels in rat endometrium during the estrous cycle. During pre-implantation, both receptors were expressed in the luminal epithelium and glandular epithelium on Days 0.5 and 1.5 of pregnancy, but were down-regulated on Days 2.5 and 3.5. During peri-implantation, both TGF-β receptors were localized in the luminal epithelium and subepithelial stroma to facilitate attachment reaction and trophoblast invasion. They were highly expressed on Day 4.5, whereas were down-regulated on Days 5.5 and 6.5. Semi-quantitative RT-PCR analysis confirmed the data obtained by in situ hybridization. These results suggest that during pre-implantation, both TGF-β receptors are functional in the proliferation of endometrial epithelial cells. During peri-implantation, both TGF-β receptors play important roles during the onset of the uterine receptivity and the attachment reaction. TGF-β signaling is down-regulated when trophoblast invasion begins.

Keywords: TGF-β receptor; Rat; Estrous cycle; Implantation; Endometrium

Introduction

Transforming growth factor (TGF)-β is a multifunctional polypeptide that regulates cellular proliferation, differentiation, migration, and apoptosis in an autocrine or paracrine fashion. The ligands of TGF-β superfamily, consisting of TGF-β isoforms (TGF-β1, 2, 3), activins, inhibins, bone morphogenic proteins (BMPs), and others, transmit their signals via transmembrane serine/threonine kinase receptors (type I and type II). Upon ligand binding, type II receptor recruits and phosphorylates type I receptor, followed by the activation of the Smad pathway which serves as the intracellular signaling pathway of the TGF-β superfamily (Massague, 1998). Receptor-regulated Smads (R-Smads) are directly activated by type I receptor, form oligomeric complexes with common-partner Smads (Co-Smads, Smad4), and translocate into the nucleus where they regulate gene transcription. Among R-Smads, Smad2 and Smad3 respond to TGF-βs and activins, whereas Smad1, Smad5, and Smad8 transmit BMPs signals (Massague, 1998).

It is becoming increasingly clear that TGF-β is involved in cellular proliferation and differentiation, extracellular matrix modification, tissue remodeling, angiogenesis, and decidualization of uterine endometrium during the estrous cycle and implantation. In support of this contention, it has been shown that TGF-βs are localized at the special compartments of the endometrium during mouse pre- and peri-implantation period (Tamada et al., 1990; Das et al., 1992), and rat estrous cycle and peri-implantation (Chen et al., 1993). Localizations of TGF-βs in the endometrium of other species (Graham et al., 1992; Chegini et al., 1994; Lennard et al., 1995; Dore et al., 1996; Munson et al., 1996; Gupta et al., 1996, 1998a,b; Ando

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et al., 1998) are also consistent with their roles during the estrous/menstrual cycle and early pregnancy. Some evidence is also accumulating that TGF-β receptors are present in the endometrium during monkey/human menstrual cycle and early pregnancy (Chegini et al., 1994; Sachdeva et al., 2001), porcine peri-implantation (Gupta et al., 1998b), and mouse post-implantation (Roelen et al., 1994). However, the roles of TGF-β receptors in the estrous cycle and implantation of rodents are still currently unknown. Regarding the roles of TGF-β receptors types I and II in determining TGF-β response, identification of the endometrial distribution profiles of TGF-β receptors is an important research task for verifying the functional bioactivity of TGF-β in the estrous cycle and pregnancy.

Therefore, in the present study the expression patterns of TGF-β receptors types I (TβRI) and II (TβRII) at mRNA levels were investigated in rat uterus during the estrous cycle, pre-, and peri-implantation using in situ hybridization and semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR).

Materials and methods

Animals and tissue preparation

Adult Sprague–Dawley rats weighing 200~250g were bred under a controlled environment (temperature maintained at 25 °C, lights on from 07:00 to 19:00 h, with free access to food and water) in Experimental Animal Center of Institute of Zoology, the Chinese Academy of Sciences. To set up mating, a fertile male rat was caged with three virginal rats overnight, and the vaginal plug was checked between 08:00 and 09:00 h the next morning. Midday on the day that a vaginal plug was found was designated as Day 0.5 of pregnancy. Pregnant rats were killed on Days 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, and 6.5 ($n=3$ for each time point). Tissues also included virgin uteri selected for stage of the estrous cycle ($n=3$ for each stage). Uteri were immediately removed and embedded in embedding medium (Triangle Biomedical Sciences, Durham, NC) at −20 °C. Sections (10-μm-thick) were cut transversely and mounted onto poly-L-Lysine-precoated slides for in situ hybridization. Animal care and treatment were performed under a protocol approved by the Animal Care and Use Ethical Committee, State Key Laboratory of Reproductive Biology, Institute of Zoology, the Chinese Academy of Sciences.

Plasmid constructs and probe production

A 743- and 698-bp fragments of rat TβRI and TβRII cDNA, respectively, were generated by PCR amplification of reverse-transcribed RNA using the following primers as previously reported (Kim et al., 1996): for TβRI, the 5′ primer was ACG TTC ATG GTT CCG AGA GG and the 3′ primer was TCG CAA AGC TGT CAG CCT AGC; for TβRII, the 5′ primer was AAG TCT TGC ATG AGC AAC TGC and the 3′ primer was GAT GTC AGA GAA GAT GTC C. The PCR products were purified from the agarose gel using NucleoTrap Gel Extraction Kit (Clontech Laboratories Inc., Palo Alto, CA). Amplified TβRI sequences were T–A cloned into the pMD-18T vector (TaKaRa, Dalian, China). Further *Kpn*I restriction digestion was conducted to confirm the orientation of the insert. The *Eco*RI–*Sal*I fragment of TβRI-pMD-18T (TβRI insert was in reverse orientation) was cloned into pGEM-4Z vector (Promega, Madison, WI). The resulting TβRII fragment was T–A cloned into pGEM-T-easy vector (Promega) according to the manufacturer's protocol. The authenticities of the products were confirmed by sequencing (Sangon Corp., Shanghai, China). To synthesize TβRI antisense cRNA probe, the TβRI-pGEM-4Z plasmid was linearized with *Eco*RI and transcribed using digoxigenin (DIG) RNA labeling kit (T7) (Roche Diagnostics Ltd., Indianapolis, IN). TβRI sense probe was generated using *Sal*I-linearized plasmid and DIG RNA labeling kit (SP6) (Roche). TβRII antisense probe was synthesized from *Nde*I-linearized TβRII-pGEM-T-easy plasmid using T7 RNA polymerase; TβRII sense probe was generated using *Nco*I and SP6 RNA polymerase. The yields of the probes were determined using spot test following the instructions of the manufacturer with a DIG-labeled RNA control provided in the labeling kit. Spot intensities of the control and the probes were quantified using Band Leader version 3.0 (Magnitec Ltd., Tel-Aviv, Israel).

In situ hybridization

In situ hybridization was carried out as previously described (Lin et al., 2004). Briefly, the cryosections were fixed in 4% paraformaldehyde for 15 min, incubated in PBS containing 0.1% active diethylpyrocarbonate (DEPC) twice for 15 min each, and in 5 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate) for 15 min. The slides were prehybridized in prehybridization solution (50% deionized formamide, 5 × SSC, 120 μg/ml salmon sperm DNA) for 2 h at 50 °C, and hybridized in prehybridization solution containing denatured probes at a final concentration of 400 ng/ml for 18 h at 50 °C, followed by serial wash in 2 × SSC at room temperature for 30 min, in 2 × SSC at 65 °C for 1 h, and in 0.1 × SSC at 65 °C for 1 h. The slides were then incubated with anti-DIG-alkaline phosphatase (diluted 1 : 5000 in blocking solution [0.5% blocking reagent in 100 mM Tris, 150 mM NaCl, pH 7.5]; Roche) at room temperature for 2 h, and further rinsed in washing buffer (100 mM Tris, 150 mM NaCl, pH 7.5) twice for 15 min each. Color development was performed using nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP; Roche). The sense probes were used as negative controls for background levels.

Relative gene expression levels measured by semi-quantitative RT-PCR

Total RNA was extracted from freshly harvested rat uterus ($n=3$ for each time point or each stage) using Trizol reagent (Invitrogen, Gaithersburg, MD) and was removed of genomic DNA contamination by RNase-free DNaseI (Roche). Reverse transcription was carried out according to the method provided